LONG-TERM GOALS

The primary goal of these studies was to investigate the relative vulnerability of marine mammals to acoustically mediated trauma from emboli formation. By evaluating key environmental, behavioral and physiological factors, we have been able to identify drivers influencing the dive response of cetaceans and pinnipeds, and the potential risk for tissue damage at depth. The results of this project are currently being used to develop environmentally sensitive schedules for oceanic acoustic activities that take into account the underlying biological mechanisms that control physiological homeostasis in deep and shallow diving marine mammals.

OBJECTIVES

To accomplish these goals we focused on three key questions:

1. **Environmental:** *Does elevated environmental temperature compromise the dive response that safeguards marine mammals from decompression illness?* This was tested by measuring cardiovascular and metabolic parameters of marine mammals during sedentary and active periods in warm and cold water.

2. **Behavioral:** *Do increased levels of tissue globins correspond to increased plasticity of the dive response during voluntary activity by marine mammals?* Here we evaluated the physiological significance of elevated globin levels that occur in the cerebral cortex and muscles of marine mammals (Williams *et al.*, 2008). Specifically, we compared behaviorally induced variability in the dive response (as manifested by changes in the level of bradycardia and peripheral circulation) in deep and shallow diving mammal species varying in globin deposition patterns.
3. **Physiological**: *Does the timing of globin deposition and metabolic demands alter aerobic dive limits during different developmental stages in marine mammals?* By comparing both globin deposition profiles from carcasses ranging in age from neonates to adults, as well as the change in mass-specific metabolic demands across age classes, we have been assessing how age influences neuroprotective mechanisms in a wide variety of marine mammal species.

Together these studies are enabling us to determine why some marine mammal species, such as the family of beaked whales, appear more susceptible to non-auditory tissue damage as may occur in conjunction with navy and oil exploration sound operations. We take into account several recent hypotheses regarding emboli formation (e.g., Houser *et al.*, 2001; Jepson *et al.*, 2003), observed behavioral responses of marine mammals to low- and mid- frequency sound disturbance (DeRuiter *et al.*, 2013; Goldbogen, *et al.*, 2013), as well as the results of our studies to develop predictive models for susceptibility to decompression illness.

**APPROACH**

This study used two approaches to determine the relative susceptibility of different marine mammal species to acoustically mediated trauma, 1) molecular and biochemical evaluation of neuroprotection at the tissue level, and 2) whole animal/physiological assessments to determine the impact of behavioral and environmental challenges to the dive response. Laboratory studies at the tissue level assessed the presence and function of oxygen binding circulating (hemoglobin) and resident (cytoglobin, and neuroglobin) globin proteins in the brain, and myoglobin in locomotory muscles. A survey of shallow and deep diving species enabled us to determine the effects of routine dive capacity on the expression of these globins (Williams *et al.*, 2008). Our current studies built on this foundation to evaluate how age, environmental temperature and coincident metabolic demands influenced globin deposition and the avoidance of tissue damage from hypoxia in marine mammals. Ultimately, this will allow us to determine if specific segments of marine mammal populations are more susceptible than others to injury.

Team members included specialists in morphology and pathology of marine mammals (M. Miller, CA Dept. Fish and Game; D.A. Pabst, Univ. North Carolina-Wilmington), globin chemists (D. Kliger and R. Goldbeck, UCSC), molecular biologists (M. Zavanelli, UCSC) and physiologists (T.M. Williams, D. Casper, N. Thometz, R. Dunkin, and S. Noren, UCSC.)

The second component of this study examined the susceptibility of marine mammals to decompression illness at the whole animal/physiological level by monitoring behaviorally induced variability in the dive response. Because nitrogen transfer and decompression illness are linked to tissue perfusion, relaxation of the dive response in marine mammals has the potential to increase susceptibility to neural tissue damage either by preventing the removal of nitrogen or altering the perfused tissue pool available for nitrogen dispersal. The effects of exercise, an important physiological mechanism known to alter blood flow, was investigated and compared for shallow (bottlenose dolphin) and deep (Weddell seal) diving species. Dolphins were trained to dive and exercise at depths varying from 3 to 20 m. Variability in bradycardia and peripheral vasoconstriction were subsequently monitored as the animals performed sedentary to high intensity exercise tasks. These results were compared to dolphins performing extreme dives maximally to 60 - 210 m (Williams *et al.*, 1999), and to a free-ranging deep diver, the Weddell seal that foraged at >400 m.
Team members for this part of the program included physiologists (T.M. Williams, S. Noren, and L. Yeates from UCSC; R. Davis, Texas A&M University) and animal behaviorists (T. Kendall and B. Richter, UCSC; P. Berry, EPCOT)

**WORK COMPLETED**

The major effort of this research program examined variability in the dive response of marine mammals due to exercise at depth. The initial challenge was in developing instrumentation for heart signal detection that could withstand the rapid swimming movements of dolphins (Fig. 1). We have successfully developed, tested and collected data using a new submersible electrocardiograph/accelerometer monitor in collaboration with UFI, Inc. (Morro Bay, CA) and O’Neill wetsuits (Santa Cruz, CA). Ten dolphins, two beluga whales, four Weddell seals and two sea otters have been examined. Heart rate during surface and submerged resting periods were collected for all four species, including an evaluation of the effects of body position on bradycardia in dolphins. Comprehensive tests were conducted on the dolphins and Weddell seals. We completed a series of exercise tests at four different performance intensities for dolphins freely-diving to 3 m, 10 m and 20 m and Weddell seals diving to >400 m. Studies involving additional species are ongoing.

![Figure 1. Equipment for monitoring electrocardiographic (ECG) signals and stroke frequency in marine mammals. The inset to the right shows the housing, electronics and electrode pad assembly for the instrument (16 cm long x 3 cm diameter tube). The photo on the left illustrates instrument deployment on a dolphin during ascent from a shallow dive. The instrument is placed in a neoprene pocket near the sternum of the animal.](image)

Extensive analysis of the electrocardiographic data involved statistical testing for the effects of, 1) species and coincident aerobic dive limits, 2) exercise intensity as monitored by stroke frequency, 3) maximum depth and instantaneous position in the water column, 4) behavior, 5) stroke gait, and 6) tissue oxygen stores provided by globin proteins. Initial analyses developed new models of dive
responses for marine mammals taking into consideration the variation in bradycardia that occurs with
different levels of activity (Noren et al., 2013; Davis and Williams, 2013). Subsequent evaluation
added in the effect of position in the water column as a factor (Williams et al., in review). Specifically
we related variation in the dive response and the incidence of cardiac arrhythmias to the level of
exercise performance and dive depth. This is being used to formulate new hypotheses regarding
variability in the dive response and triggers for cardiac instability in marine mammals. Comparative
studies on beluga whales and sea otters are continuing.

In addition to these cardiovascular studies, we examined the effects of animal age and environmental
temperature on the diving response and tissue globin deposition in marine mammals. Our team
improved on current assays for muscle myoglobin assessment, and successfully developed two assays
for brain globins, a spectrophotometric test that provided total globin concentration and an mRNA
expression test for relative cytoglobin and neuroglobin levels. We used these assays to detect the
presence and concentration of globins in the cerebral cortex of 16 species of mammals classified
according to style of swimming and routine diving depth (Williams et al., 2008). Studies concerning
the effects of temperature and age on globin deposition and impact on aerobic dive limit were
completed for a marine mammal species varying from tropical (Williams et al., 2011a) to temperate
(Thometz et al., 2011 and in review; Liwanag et al., 2012a, b) to polar (Williams et al., 2011b)
thermal habitats. We have also collected data on the combined effects of exercise and increased
environmental temperature on cardiovascular responses in dolphins using skin heat flow and changes
in core body temperature as metrics for alterations in blood flow. Data analysis for this portion of the
program is ongoing and includes cardiovascular signatures for different levels of exercise intensity and
water temperature.

The results from these studies were presented in a series of talks and posters at the Society of Marine
Mammalogy meeting (Tampa FL, November 2011) and talks at the Society for Integrative and
Comparative Biology meeting (Seattle WA, 2010; Charleston SC, January 2012; San Francisco CA,
2013) and the International Mammological Conference (Belfast, Ireland, August 2013). Predictive
models of gas movement in the cardiovascular system, aerobic dive limits, and susceptibility to
decompression illness based on our results were discussed at the Diving Marine Mammal Gas
Kinetics Workshop (Woods Hole MA, April 2010) and were incorporated into peer-reviewed
manuscripts listed below.

RESULTS

The most important finding from this project was that the dive response of marine mammals is far
more complex than previously known. We first demonstrated the effect of exercise intensity on diving
bradycardia by simultaneously monitoring both heart rate and stroke frequency of marine mammals
diving to different depths under both controlled and free-ranging conditions (Fig. 2). In contrast to the
common description of diving “reflexes” in marine mammals, both dolphins and Weddell seals
demonstrated a release from bradycardia with increased locomotory effort. Preferred speeds and gait
patterns (gliding, stroke and glide, constant stroking) selected by the animals modified the response.
Dolphins showed a 2.2-fold increase in heart rate from submerged rest to 72 strokes.min⁻¹ at preferred
swimming speeds; seals showed a 3.8-fold change in heart rate over the range of speeds (Davis and
Williams 2012; Noren et al., 2012). This is a marked departure from the relatively constant
bradycardia believed to occur at depth.
Subsequent experiments and data analyses revealed an interactive effect between exercise intensity and dive depth on these cardiac responses. For both test species the level of bradycardia declined as the animals moved through the water column (Williams et al., in review), with the greatest rate of change occurring at shallow depths typically associated with lung collapse (Fahlman et al., 2009). Based on these data, we hypothesize that the heart of active marine mammals receives graded, conflicting autonomic neural signals during submerged activity. This originates from the balance between sympathetic signals which act to increase heart rate with exercise, and parasympathetic signals which mediate suppression of heart rate in diving marine mammals. We attribute cardiac arrhythmias (ectopic beats, inter-beat interval variability) observed during stroke gait transitions to this proposed autonomic neural conflict. Furthermore, the incidence of these cardiac arrhythmias appears to be species-specific and related to maximum depth of the dive (Williams et al., in review).

Figure 2. The correlation between heart rate and stroke frequency in diving bottlenose dolphins (a) and Weddell seals (b). The top figure shows the electrocardiogram waveform for heart rate and corresponding 2-axis accelerometer traces representing individual strokes of the dolphin flukes. Similarly, the bottom figure illustrates the correspondence between seal flipper stroke frequency and heart rate by superimposing both parameters on the time-depth profile of a single dive to 135 m. Colors indicate the range of values for heart rate in beats.min\(^{-1}\) and stroke frequency in strokes.min\(^{-1}\). (From Davis and Williams, 2012.)
The proposed mechanism for cardiac control in diving marine mammals has marked implications for physiological stability and the mobilization of gases during submergence, especially during prolonged, high speed or deep dives. Our data indicate that specific behaviors or segments of a dive are riskier than others in terms of inducing cardiac instability. From our free-ranging studies we find that marine mammals tend to maintain preferred levels of exercise which result in only modest changes in the dive response. This not only shapes foraging tactics, but likely contributes to the exceptional sensitivity of some marine mammal species (e.g., deep diving beaked whales) to anthropogenic-derived acoustic disturbances by altering swimming speed, ascent rate, and stroke frequency.

Lastly, companion studies conducted during this project have added new insights concerning thermal (Fig. 3), tissue globin, and age-related effects on the diving capabilities in marine mammals and are detailed in the publications list at the end of this report.

Figure 3. The effect of acclimation temperature on resting metabolic rate (RMR) of mammals. Terrestrial species (green), tropical seals (red), temperate and polar phocid seals (black), and cetaceans, otariids, and sea otters (blue) are compared. Data for adults (circles) and immature animals (triangles) are shown. Lines represent the least squares linear regressions for RMR in relation to body mass as described in Williams et al. (2011a).

**IMPACT/APPLICATIONS**

Our recent findings on variability in the cardiovascular response to diving and in tissue globin levels provide:

1. **A new perspective on physiological stability in deep diving mammals.** By examining a wide variety of mammalian species living in different habitats, we demonstrated a heretofore unknown malleability in the diving response of marine mammals. Both position in the water column and level of exercise were found to alter the level of bradycardia achieved during submergence (Williams et al., in review). Furthermore, the marine mammal heart showed a level of instability that was surprisingly similar to that of humans during accidental cold water submersion (Shattock and Tipton, 2012). Because the instigators for this instability are similar to those associated with flight responses of cetaceans exposed to anthropogenic noise (DeRuiter et al., 2013; Goldbogen et al., 2013), these data
provide an important link between disturbance, behavior and potential for tissue injury during diving. These data are now being used by our team and others to create new models of gas mobilization (i.e., Davis and Williams, 2012) that will define species-specific robustness or vulnerability to anthropogenic disturbance.

2. **An assessment of the importance of globin proteins.** Since neuroglobin and cytoglobin have been associated with neuronal survival following stroke and other ischemic insults with cardiovascular accidents, the results are relevant to many of the leading causes of mortality in the United States. Likewise, species-specific differences in resident neuroglobins may also help to explain the relative susceptibility of deeper diving species to barotrauma following exposure to anthropogenic noise.

3. **New techniques for clinical, ecological, behavioral and physiological studies.** The instrumentation developed for simultaneously monitoring electrocardiographic signals and swimming stroke performance in freely-diving marine mammals and the predictive models being tested provide new tools for assessing the response of wild mammals to anthropogenic disturbance. In addition, our study is developing new biochemical methods and animal models for the evaluation of brain globins that should be of interest to a wide variety of comparative and medical neurophysiologists.

**RELATED PROJECTS**

A recent award by ONR (High Risk Behaviors in Marine Mammals: Linking behavioral responses to anthropogenic disturbance to biological consequences, # N00014-13-1-0808) will continue these studies and enable all of the results to be compiled into stochastic dynamic state variable (SDSV) and Population Consequences of Acoustic Disturbance (PCAD) models. These models will enable us to make definitive links between species-specific vulnerabilities, population dynamics of divers and acoustic disturbance in oceans. As such, they will improve our ability to predict lethal and non-lethal consequences of noise on marine living mammals.

**REFERENCES**

(Note: References with the journal title in bold are from this project with a full listing according to date of publication below)


PUBLICATIONS AND PRESENTATIONS


The Society for Marine Mammalogy Abstracts. Tampa FL, November 26 - December 2, [published, refereed].
